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MORPHOLOGICAL AND PHOTOSYNTHETIC RESPONSES OF IN VITRO CULTURE OF INDIA ECHINACEA (*Andrographis paniculata*) TO LIGHT SPECTRUM AND CARBON SOURCES

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Abstract — *Andrographis paniculata* (Burm.F.) Wall ex. Nees is an important medicinal plant in ASEAN countries that drives a high demand in the worldwide medicinal plant trade due to its andrographolide content. To find out optimum lighting and culture conditions *in vitro*, seed cultures were grown on photoautotrophic (sucrose- free) and photomixotrophic (3% sucrose) media. Blue and red monochromatic LED lights were used at an irradiance intensity of 40 µmol m^{-2} s^{-1} for 20 days as an energy source. Photosynthetic responses were measured in terms of chlorophyll content and net photosynthetic rate. Morphological responses were measured in terms of leaf and internode count, shoot length, number of roots, root length, fresh weight, and dry weight. Results were analyzed using SPSS 11.5 software. Gas chromatography results show that net photosynthetic rate was higher under blue light while red light resulted in taller plants. Root count and root length were higher under red light by 11% and 9.7% respectively. Sucrose reduced the net photosynthetic rate under both light spectra. Furthermore, plants under photoautotrophic conditions grew taller shoots and higher fresh weight. However, photomixotrophic blue light plants showed the highest dry matter content. Spectrum- wise, red light plants have a higher dry weight but when sucrose was involved in the growing medium, blue light plants outweighed it by as much as 7.91%. This suggested hyperhydric tissues in other cultures. Photosynthesis is constrained often by the low concentration of CO_2 in photomixotrophic cultures as sucrose has been observed to act as an osmotic agent in *in vitro* culture systems.

Keywords — ***Andrographis paniculata*, photosynthesis, red light, blue light, sucrose**

INTRODUCTION

Photosynthetically active radiation (PAR) drives the photosynthetic process in a spectrum-specific effect. Blue and red monochromatic lights have effects both on photosynthesis and morphogenesis and are the more photosynthetically effective spectra (McCree,1972; Inada, 1976). Among the effects of red light is stem elongation (Yanagi,1996) while blue light favors chlorophyll formation (Humbeck et al., 1984) and more efficient photosynthesis. Nearly all of the carbon and chemical energy needed for plant growth is provided during photosynthesis (Björkman, 1981). However, plants can also utilize soluble sugars as a source of carbon. Hdider et al (1993) reported the photosynthetic influence of sucrose in plants. Sucrose also influenced in vitro plant organogenesis (Arigita et al., 2002).

These findings are the fundamentals in investigating the effects of the light spectrum and sucrose as carbon sources on the medicinal plant *Andrographis paniculata. A. paniculata* (Burm. f.) Wall ex. Nees is an important medicinal plant in ASEAN countries (Akbar, et. al., 2011; Kabir, et al., 2014) that drives a high demand in the worldwide medicinal plant trade (Hossain et al., 2014) due to its andrographolide content (Shah et al., 2007). *In vitro* culture of this plant could result in maximizing andrographolide production.

However, optimum conditions for effective in vitro production must be known thus, the effects of red and blue light and sucrose as a carbon source on *Andrographis paniculata* were examined.

MATERIALS AND METHODS

Plant Materials and Treatments

Prior to germination, seeds of *A. paniculata* were surface-sterilized with 20% Clorox (The Clorox Co., Oakland, CA, USA) and mechanically shaken for 20 minutes. Surface- sterilized seeds were then grown on Murashigue and Skoog (1962) medium under white fluorescent light before treatment application. The culture medium was pH- adjusted to 5.7 before autoclaving. Thirty-day- old plants were then aseptically transferred to Phytagel-solidified MS medium inside 250 mL glass vessels for photoautotrophic and photomixotrophic growth. Photomixotrophic medium contained 3% sucrose as a source of carbon. Ambient CO_2 was utilized as a carbon source for photoautotrophic growth. The lid of photoautotrophic vessels was perforated and covered with gas-permeable microporous polypropylene film (0.22 µm pore size) to allow air exchange. Monochromatic blue and red LED lights were used to subject the plants under different PAR. LED lights were set at 40 µmol m-2 s-1 of photosynthetic photon flux (PPF) for 20 days.

Morphology Parameters, Fresh and Dry Weights

A number of leaves were counted during days 0, 10, and 20 of the experiment. The final leaf count was done on day 20. Shoot length was measured from the crown of the plant up to the terminal bud. The shoot and root length of the test plants were measured after taking them out of the growing vessel. The length was measured from the belowground base of the plant up to the tip of the primary root. Shoot length was measured on days 0, 10, and 20 of the experiment. The number of internodes was counted through visual recognition on day 20.

Fresh weight of the whole plant was measured using an Adventurer OHAUS ARC 120 2- point balance. Plants were removed from the growing bottle and cleaned off of residual MS medium that attached to the plant roots. The dry weight of the whole plant was measured using an Adventurer OHAUS ARC 120 2- point balance. The whole plant parts used for measuring fresh weight were cut into stem, leaf, and root components before being put into aluminum foil packets. Samples were then oven-dried using Hot Air Oven at 65° C for 72 hours. Samples were then allowed to cool at room temperature before being weighed.

Net Photosynthetic Rate and Chlorophyll Content

The net photosynthetic rate was measured by reference to the internal and external concentrations of CO_2 within and outside the growing bottles. This was quantified through gas chromatography using Shimadzu GC Model GC- 17A. The net photosynthetic rate was then computed using the formula of Fujiwara, et. al., (1987) given as [Pn] = K x E x V (Cout - Cin)/ Leaf Area; where K is a conversion factor converting CO_2 amount from volume to mole (40.5 mol m-3 at 28° C) E is the number of air exchanges per hour (2.32 h-1) and V is the air volume of the growing vessel (0.0025 m3) (Fujiwara et al., 1987). Non- destructive chlorophyll measurement was done using SPAD 502 Chlorophyll Meter. Measurement was taken on the first or second leaf of the sample plants by attaching the probe onto three different sections of a leaf. The average of the three readings is interpreted as the instantaneous chlorophyll content.

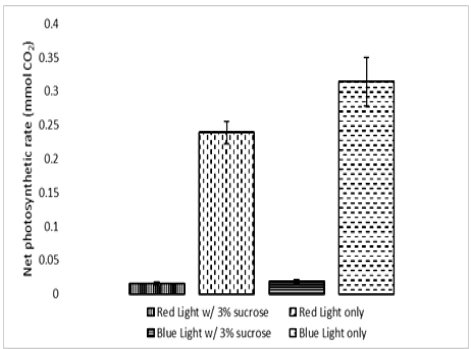
Data Analysis

Data were analyzed using SPSS Version 11.5. The data were run through a Two-Factor Analysis of Variance (ANOVA). Significant differences among means were calculated through Duncan's Multiple Range Test.

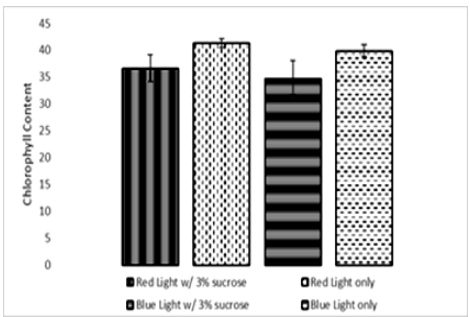
RESULTS AND DISCUSSION

The net photosynthetic rate was lower among photomixotrophic vessels (Figure 1-a). Plants in this culture suffered as much as a 94% reduction in their net photosynthetic rate regardless of the light spectrum. It was observed that leaves under photomixotrophic culture showed more folds and curls. Spectrum-wise, red light plants assimilated 13-17% lower while blue-light plants showed relatively higher assimilation. Moreover, regardless of cultural conditions, blue light plants show to be more photosynthetically efficient than the red- light counterparts. Although chlorophyll content data (Figure 1-b) might show red-light plants to probably have more roots that light-harvesting complexes (LHCs), variance analysis shows no significant chlorophyll content difference. Chlorophyll content, however, was 16% higher in red-light photoautotrophic conditions and 5% higher in red-light photomixotrophic conditions than on the blue light cultures.

Number of internodes was not affected (Figure 2-a) but showed to have possible direct correlation to shoot length. (Figure 2-b). However, shoot length was affected being taller under photoautotrophic conditions. Although insignificant, an inverse relationship was observed where under photoautotrophic conditions, blue light triggered a 7% increase in shoot length but in photomixotrophic conditions, red light plants were 13% taller. Root length (Figure 2-d) and the number of roots (Figure 2-c) were affected by red light. There were 11% more were 9.7% longer on red light plants. Although insignificant, a spectrum-restricted observation was noted on red light



1-a



1-b

Fig. 1. Photosynthetic responses of *Andrographis paniculata* grown under *photomixotrophic* and photoautotrophic condition *In vitro*; net photosynthetic rate (Figure 1-a) and chlorophyll content (Figure 1-b).

plants where the red light-triggered increase in root count under photomixotrophic conditions. On the other hand, red light promoted longer roots under photoautotrophic conditions. Unlike blue light where its effects were only on shoot length, red light encompassed both root count and root length.

In terms of weight, fresh weight (Figure 2-e) was higher among red light plants than those grown on blue light. It was lowest on photomixotrophic blue light conditions and highest on photoautotrophic red light conditions. However, photomixotrophic blue light plants showed the highest dry matter content. Spectrum-wise, red light plants have a higher dry weight but when sucrose was involved in the media, blue light plants outweighed it by as much as 7.91%.

Plant development and physiology are strongly influenced by the light spectrum of the growth environment (Hogewoning et al,2010) and different wavelengths in the PAR have specific effects on the plant. For in vitro cultured A. paniculata, net photosynthesis was highest among plants grown under blue light in sugar- free medium. Blue light is involved in a wide range of plant processes such as phototropism, photomorphogenesis,stomatal opening and leaf photosynthetic functioning (Whitelam and Halliday, 2007). Moreover, blue light has been associated with the expression of 'sun-type' characteristics such as a high photosynthetic capacity at the chloroplast level, (Lichtenthaler et al., 1980).

A lower photosynthetic rate under red light has been observed in many plant species (Matsuda et al., 2004; Goins et al., 1997) than those plants grown under the presence of blue light. Long-term exposure to monochromatic red light can result in photosynthetic dysfunctional leaves characterized by a suboptimal and heterogeneously distributed dark-adapted Fv/Fm, a stomatal conductance unresponsive to irradiance, and a relatively low light-limited quantum yield for CO2 fixation (Hogewoning et al., 2010). Photosynthesis is constrained often by the low concentration of CO2 in photomixotrophic cultures (Kubota, 2002). The decrease in Pn could also be attributed to lower chlorophyll content. Mohamed and Alsadon (2009) showed that the total chlorophyll content was higher when plants were grown in ventilated vessels than in non-ventilated vessels.

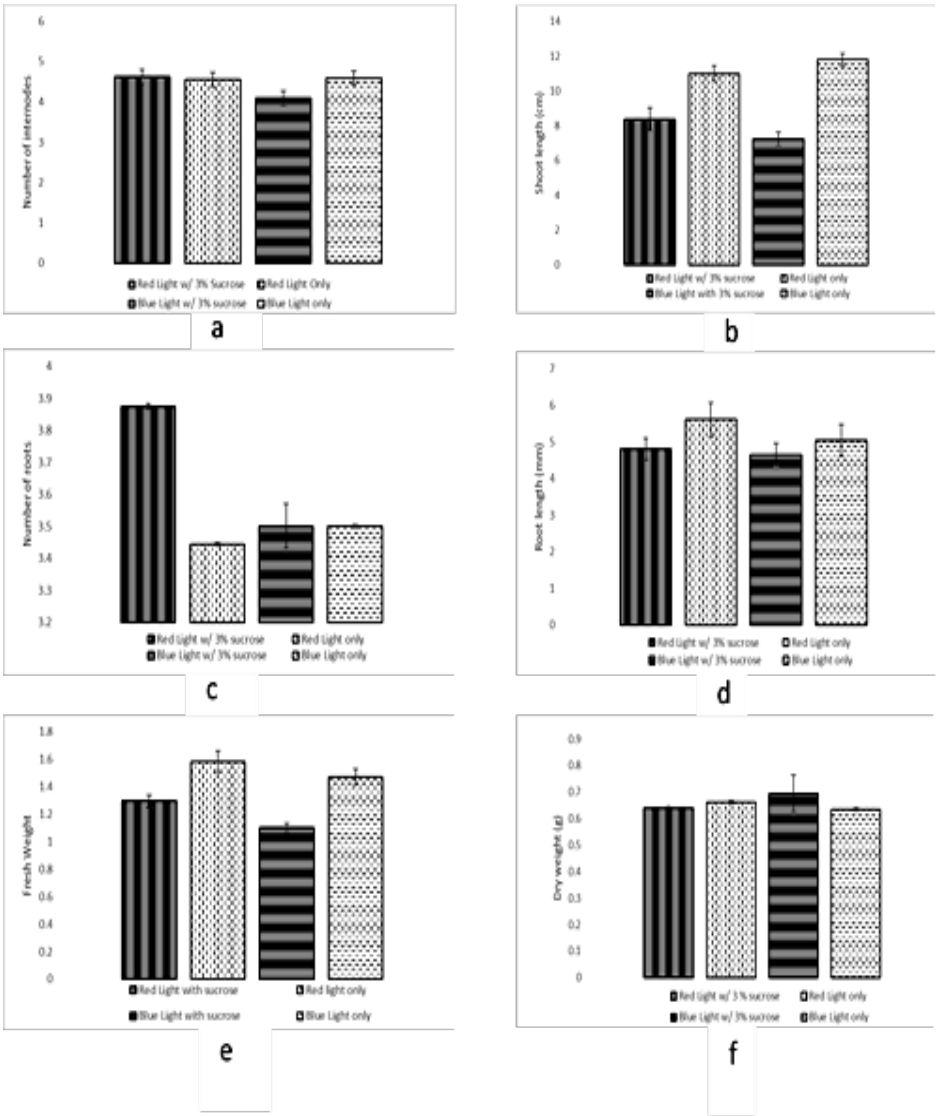


Fig. 2. *Andrographis paniculata* cultured on photomixotrophic and photoauto trophic conditions under blue and red light. (Fig. 2a. Number of inter nodes; Fig. 2b. Shoot length; Fig. 2c. Number of roots; Fig. 2d. Root length; Fig. 2e. Fresh weight, and Fig. 2f. Dry weight).

Red light has been frequently shown to affect plant morphogenesis (Fukuda et al., 2011) which stimulates shade avoidance response in plants such as enhanced stem elongation (Smith, 1995). Highest shoot length was observed on plants grown under red light which coincides with this finding but it does not differ significantly with those grown under blue light. Blue light irradiance has been found to inhibit hypocotyl elongation (Hoenecke et al., 1992) and the reduction of blue light increased shoot length (Mortensen and Stromme, 1987) while red light irradiance resulted into longer shoots (Fukuda et al., 2011). Chlorophyll plays a critical role in the process of

photosynthesis. Changes in its level have been used to evaluate photosynthetic activity and changes in the proportion of chlorophyll *a* to chlorophyll *b* have been used as a marker for tolerance to abiotic stresses in plants (Larcher, 1995). Hassankhah et al, (2014) showed that plants grown in ventilated vessels had significantly higher chlorophyll content. The exogenous supply of sucrose which is not required for the normal development of photosynthetic apparatus normally produces low chlorophyll content in *in vitro* plants (Grout and Donkin, 1987; Mohamed and Alsadon, 2009) but this decrease is not significant as was observed by Cui et al., (2000). Although blue light has been reported to likely have more chlorophyll content (Hogewoning et al., 2010), the red light spectrum has been shown to induce more chlorophyll pigments in *Andrographis paniculata.*

Root formation is influenced by red light (Gabryszewka and Rudnicki, 1997). However, this result was only obtained when the red light is applied in combination with an exogenous auxin (Rossi et al., 1993). The results of Rossi et al. (1993) could be explanatory of the findings in this study that neither red nor blue light spectrum resulted in higher root length. However, Rossi et al. (1993) have observed that red light could influence root formation when in combination with auxin. This could explain why sucrose-treated plants under red light showed the highest average number of roots. Sucrose has been reported to have caused possible hormone-like effects in shoot cultures of potatoes (Vinterhalter et al., 1996). Taylor and van Staden (2001) found that *in vitro* cultured *Eucomis autumnalis* dry weight was highest in photomixotrophic cultures with 4% sucrose. The absence of osmotic stress among plants grown in a photoautotrophic medium could have encouraged hyperhydricity resulting in low dry weight. Lower dry weights of the hyper-hydrated shoots could be attributed to the high water content of the shoots (Afreen, 2007). Blue light caused a higher number of internodes contrasting the findings of Lund et al, (2007), stating that the number of internodes was determined by the red to a far-red ratio of light. But when exogenous sucrose is involved, *A. paniculata* tends to have a higher number of internodes.

In terms of fresh weight, several previous findings on *in vitro* cultures were confirmed by the observations in *A. paniculata*. In the studies of Kozai et al. (2002) and Rahaman and Alsadon (2007), photoautotrophic cultures showed higher fresh weight than those in photomixotrophic cultures. However, on a dry weight basis, photomixotrophic blue light plants were heavier. This could suggest hyperhydric tissues in other cultures. Sucrose has been observed to act as an osmotic agent in *in vitro* culture systems (Mukherjee et al., 1991).

CONCLUSIONS

Growing seed cultures on photoautotrophic and photomixotrophic media were done to find out the optimum lighting and culture conditions in vitro. The energy source made use of Blue and red monochromatic LED lights at an irradiance intensity of 40 µmol m-2 s-1 for 20 days. Photosynthetic and morphological responses were measured and analyzed using SPSS 11.5 software.
The net photosynthetic rate was higher under blue light based on the gas chromatography results. Likewise, exposure of plants to red light produced taller plants. More roots (11%) and longer roots (9.7%) were produced under red light. Under both light spectra, sucrose was found to reduce the net photosynthetic rate. Plants were found to have taller shoots and higher fresh weight under photoautotrophic conditions.

Photomixotrophic blue light plants had the highest dry matter content. The red light plants have a higher dry weight however it was outweighed at 7.91% by the blue light but when sucrose was in the growing medium suggesting the presence of hyperhydric tissues in other cultures. Low concentration of CO_2 constrained photosynthesis in photomixotrophic cultures with sucrose observed to act as an osmotic agent.

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REFERENCES

Afreen, F. 2007. Physiological and Anatomical Characteristics of in Vitro Photoautotrophic Plants, pp 62-87. In: T. Kozai, F. Afreen and S.A.M. Zobayed (eds.) Photoautotrophic (Sugar- Free Medium) Micropropagation as a New Micropropagation and Transplant Production System. Springer Publishers, Netherlands. pp.62-87.

Akbar, S. 2011. *Andrographis paniculata*: a review of pharmacological activities and clinical effects. Alternative Medicine Review 16(1) : 66- 77.

Appelgren, M. 1991. Effects of light quality on stem elongation of *Pelargonium* in vitro. Scientia Horticulturae, Elsevier Science Publishers B.V., Amsterdam Short Communication. 45, 345-351.

Arigita, Luis, González, Aida, Sánchez Tamés, Ricardo. 2002. Influence of CO2 and sucrose on photosynthesis and transpiration of *Actinidia deliciosa* explants cultured in vitro. 115(1): 166-173. https://doi.org/10.1034/j.13993054.2002.1150119.x

Björkman, O. 1981. Responses to Different Quantum Flux Densities. In: Lange O.L., Nobel P.S., Osmond C.B., Ziegler H. (eds) Physiological Plant Ecology I. Encyclopedia of Plant Physiology (New Series) 12 . A. Springer, Berlin, Heidelberg.

Cui, Y., E. Hahn, T. Kozai, and K. Paek. 2000. Number of Air Exchanges, Sucrose Concentration, Photosynthetic Photon Flux, and Differences in Photoperiod and Dark Period Temperatures Affect Growth of *Rehmannia glutinosa* Plantlets. Plant Cell Tissue Organ Cult. 62:219-226.

Fujiwara, K., T. Kozai and I. Watanabe. 1987. Measurements of carbon dioxide gas concentration in closed vessels containing tissue cultured plantlets and estimates of net photosynthetic rates of the plantlets. J. Agr. Meteorol. 43(1):21-30.

Fukuda, N., Kobayashi-Yoshinaka, M., Ubukawa, M., Takayanagi, K. and Sase, S. 2002. Effect of light quality, intensity and duration from different artificial light sources on the growth of petunia (Petunia × hybrida Vilm.). J. Japan. Soc. Hort. Sci. 71:509-516.

Gabryszewska, E. and R.M. Rudnicki. 1997. The effects of light quality on the growth and development of shoots and roots of *Ficus benjamina* in vitro. Acta Hortic. 418:163–167.

Goins, GD, Yorio NC, Sanwo, MM, Brown, CS. 1997. Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. J. Exp.Bot. 48:1407–1413.

Grout, B.W.W. and M.E. Donkin. 1987. Photosynthetic Activity of Cauliflower Meristem Cultures and at Transplanting into Soil. Acta Hort. 212:323–327.

Hassankhah, A., Vahdati, K., Lotfi, M., Mirmasoumi, M, Preece, J. and A. Mohammad-Hasan. 2014. Effects of Ventilation and Sucrose Concentrations on the Growth and Plantlet Anatomy of Micropropagated Persian Walnut Plants. International J. Horti. Sci. and Tech. 1(2): 111-120.

Hdider, Chafik, Desjardins Yves. 1994. Effects of sucrose on photosynthesis and phosphoenolpyruvate carboxylase activity of in vitro cultured strawberry plantlets. Plant Cell, Tissue and Organ Culture. 36: 27-33.

Hoenecke, M.E., Bula, R.J. and Tibbitts,T.W. 1992. Importance of 'Blue' photon levels for lettuce seedlings grown under red-light-emitting diodes. HortScience 27:427-430.

Hogewoning, SW., Trouwborst, G., Maljaars, H., Poorter, H., van Ieperen, W., and Harbinson,J. 2010. Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. J. Exp. Bot. 61: 3107-3117.

Humbeck K., Schumann R., Senger H. 1984. The Influence of Blue Light on the Formation of Chlorophyll-Protein Complexes in *Scenedesmus*. In: Senger H. (eds) Blue Light Effects in Biological Systems. Proceedings in Life Sciences. Springer, Berlin, Heidelberg. http://doi.org/10.1007/978-3-642/60967-8_4.

Inada, K.1976. Action spectra for photosynthesis in higher plants. Plant and Cell Physiol., 17, 355-365.

Kabir, M.H., N. Hasan, M. M. Rahman. 2014. A survey of medicinal plants used by the Deb barma clan of the Tripura tribe of Moulvibazar District, Bangladesh. J. Ethnobiology and Ethnomedicine 10(1):19.

Kozai, T., Y. Koyama, and I. Watanabe. 2002. Multiplication of Potato Plantlets in Vitro with Sugar-Free Medium Under High Photosynthetic Photon Flux. Acta. Hort. 230:121-128.

Kubota, C. 2002. Photoautotrophic Micropropagation: Importance of Controlled Environment in Plant Tissue Culture. Proc. Intl. Plant Prop. Soc. 52:906– 913.

Larcher, W. 1995. Physiological Plant Ecology. Springer Verlag, Berlin, Heidelberg, 424-426.

Lichtenthaler, H, Buschmann C, Rahmsdorf U. 1980. The importance of blue light for the development of sun-type chloroplasts. In: Senger H, ed. The blue light syndrome. Berlin: Springer-Verlag, pp. 485–494.

Lund, J.B., T.J. Blom and J.M. Aaslyng. 2007. End- of- day lighting with different Red/Far-red ratios using light emitting diodes affects plant growth of *Chrysanthemum x moliforium* Ramat. 'Coral Charm'. HortSci. 42: 1609- 1611.

Matsuda, R, Ohashi-Kaneko K, Fujiwara K, Goto E, Kurata K. 2004. Photosynthetic characteristics of rice leaves grown under red light with or without supplemental blue light. Plant and Cell Physio. 45: 1870–1874.

McCree, K. J. 1972. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agric. Meteorol. 9: 191-216.

Mohamed, M.A.-H., Alsadon, A.A. (2009). Influence of ventilation and sucrose on growth and leaf anatomy of micropropagated potato plantlets. Scientia Horticulturae 123: 295–300.

Mohamed, M.H. and A.A. Alsadon . 2010. Influence of Ventilation and Sucrose on Growth and Leaf Anatomy of Micropropagated Potato Plantlets. Sci. Hort.123:295–300.

Mortensen, L.M., Stromme, E., Sebesta, Z. and Wenner, D.1987. Growth chambers with control of light quality. Norw. J. Agric. Sci., 1: 1-5.

Mukherjee, SK, Rathinasabapathi B, Gupta N . 1991. Low sugar and osmotic requirements for shoot regeneration from leaf pieces of *Solanum melongena* L. Plant Cell Tiss. Org. Cult. 25:13–16

Rahman, MD and AAJ Alsadon. 2007. Photoautotrophic and Photo-mixotrophic Micropropagation of the three potato cultivars. Bio-sci. 15: 111-116, 2007. ISSN 1023-8654 http://www.banglajol.info/index.php/JBS/index.

Rossi E Baraldi R, Facini O and Lercari B.1993. Photomorphogenic effects on in vitro rooting of Prunus rootstock GF 655-2. Plant Cell Tiss. Org. Cult. 32:145-151.

Shah, K., Trivedi, P., Shivprakash, Pundarikakshudu, K. 2007. Spectrophotometric Determination of Andropgrapholides in Andrographis paniculata Nees and its formulation. Indian J. Pharmaceutical Sci. 69 (3): 457-458.

Smith, H. 1995. Physiological and ecological function within the phytochrome family. Ann. Rev. Plt. Physio. and Plt. Mol. Bio. 46:289–315.

Smith, I.E., M.J. Savage, and P. Mills. 1984. Shading effects on greenhouse tomatoes and cucumbers. Acta Hort. 148_62: 491-500.

Taylor, JLS. and J. Van Staden. 2001. The effect of nitrogen and sucrose concentrations on the growth of *Eucomis autumnalis* (Mill.) Chitt. plantlets in vitro, and on subsequent anti-inflammatory activity in extracts prepared from the plantlets. Plant Growth Regulation.34: 49–56.

Vinterhalter, D. and B. Vinterhalter. 1996. The relationship between sucrose and cytokinins in the regulation of growth and branching in potato cv Desiree shoot cultures. Acta Horticulturae 462: 319-323.

Whitelam, G. and K. Halliday. 2007. Light and plant development. Oxford: Blackwell Publishing. 325 pp.

Yanagi, T., Okamoto, K., Takita, S. 1996. Effects of blue and blue/ red lights of two different PPF levels on growth and morphogenesis of lettuce plants. Acta Hortic. 440:117–122.

Yorio, NC., Goins GD, Kagie HR, Wheeler RM, Sager JC. 2001. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. Hort. Science 36: 380–383.